AD-A112 971 LETTERMAN ARMY INST OF RESEARCH PRESIDIO OF SAN FRANC--ETC F/G 6/20
THE MUTAGENIC POTENTIAL OF 2,4-DIAMINO-6-(2'-NAPTHYL-SULFONYL) --ETC(U)
FEB 82 L J SAUERS, J T FRUIN

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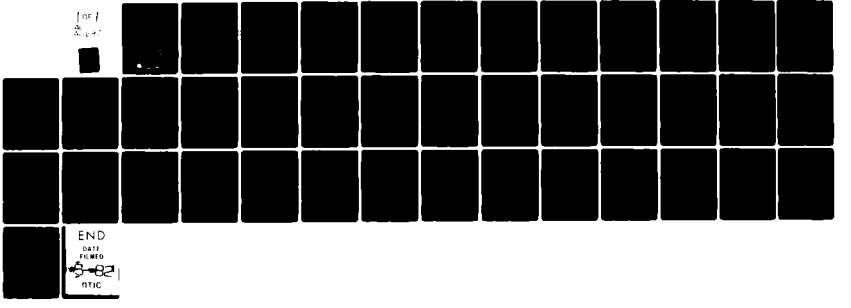

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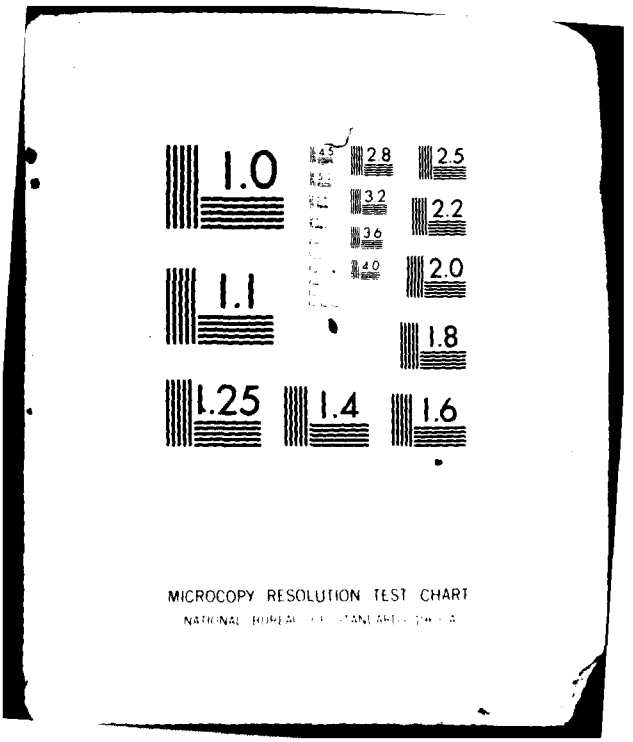
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MICROCOPY RESOLUTION TEST CHART

INSTITUTE REPORT NO. 114

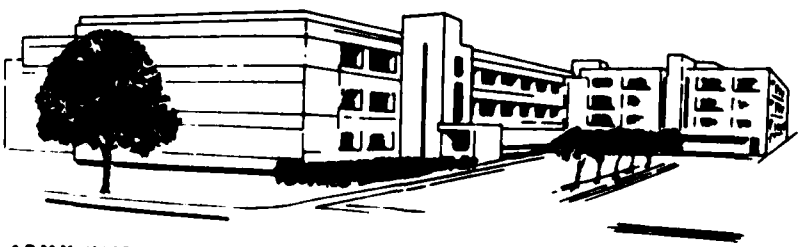
THE MUTAGENIC POTENTIAL OF:
2,4—diamino—6—(2'-napthyl-sulfonyl) quinazoline

LEONARD J. SAUERS, BA, SP5
and
JOHN T. FRUIN, DVM, PhD, COL VC

TOXICOLOGY GROUP,
DIVISION OF RESEARCH SUPPORT

FEBRUARY 1982

Toxicology Series 28



LETTERMAN ARMY INSTITUTE OF RESEARCH PRESIDIO OF SAN FRANCISCO CALIFORNIA 94129

THE MUTAGENIC POTENTIAL OF: chloroquine phosphate USP
Toxicology Series 27 -- Sauers and Fruin

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Robert Taylor COL MC 26 Feb 1982

(Signature and date)

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

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7. AUTHOR(s) Leonard J. Sauers, BA, SP5 John T. Fruin, DVM, PhD, COL VC		8. CONTRACT OR GRANT NUMBER(s)
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Mutagenicity, Toxicology, Ames Assay, 2,4-diamino-6-(2'napthyl-sulfonyl) quinazoline, WR 158122

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The mutagenic potential of 2,4-diamino-6-(2'napthyl-sulfonyl) quinazoline (WR158122*) was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Assay. Tester strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 were exposed to doses ranging from 10^{-3} mg/plate to 3.2×10^{-7} mg/plate. It was determined that the test substance did not have mutagenic potential.

*Code Number for the Compound

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ABSTRACT

The mutagenic potential of 2,4-diamino-6-(2'-napthyl-sulfonyl) quinazoline (WR158122*) was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Assay. Tester strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 were exposed to doses ranging from 10^{-3} mg/plate to 3.2×10^{1} mg/plate. It was determined that the test substances did not have mutagenic potential.

* Code number for compound.

PREFACE

TYPE REPORT: Ames Assay GLP Study Report

TESTING FACILITY: US Army Medical Research and Development Command
Fort Detrick, Frederick MD 21201

Letterman Army Institute of Research
Presidio of San Francisco, CA 94129

SPONSOR: US Army Medical Research and Development Command
Division of Experimental Therapeutics
Walter Reed Army Institute of Research, Bldg 40
Washington, D.C. 20012

PROJECT/WORK UNIT/APC: 3S162734A875BD, Medical Systems in Chemical Defense, WU 302 Good Laboratory Training, APC TL07

GLP STUDY NUMBER: 81032

STUDY DIRECTOR: COL John T. Fruin, DVM, PhD, VC, Diplomate of American College of Veterinary Preventive Medicine

PRINCIPAL INVESTIGATOR: SP5 Leonard J. Sauers, BA

REPORT AND DATA MANAGEMENT: A copy of the final report, study protocol, historical data, and retired SOPs will be retained in the LAIR Archive. Test compounds were provided by the sponsor. Report on the chemical analysis appears in Appendix A.

TEST SUBSTANCE: 2,4-diamino-6-(2'-napthyl-sulfonyl) quinazoline
Vehicle - DMSO

INCLUSIVE STUDY DATES: October - December 1981

OBJECTIVE: To determine the mutagenic potential of the above compound using the Ames Assay. Tester strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 were used. The plate incorporation method was followed. The test substance was dissolved in DMSO and this diluent checked for sterility

ACKNOWLEDGMENT

The authors wish to thank John Dacey, SP4 Lawrence Mullen, BS, and SP4 Thomas Kellner, BA, for their assistance in performing the research.

Signatures of Principal Scientists Involved
In The Study

We, the undersigned, believe the study described in this report to be scientifically sound and the results and interpretation to be valid. The study was conducted to comply to the best of our ability with the Good Laboratory Practice Regulations outlined by the Food and Drug Administration.

Leonard J. Sauers 18 Feb 82

LEONARD J. SAUERS/DATE
SP5, BA
Principal Investigator

John T. Fruin 18 Feb 82

JOHN T. FRUIN, DVM, PhD/DATE
COL, VC
Study Director

DEPARTMENT OF THE ARMY
LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

REPLY TO
ATTENTION OF

SGRD-ULZ-QA

23 November 1981

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LAIR GLP study 81032 the following inspections were made:

5 October 1981
7 October 1981
9 October 1981
14 October 1981
22 October 1981

The report and raw data for this study were audited on 20 November1981.

Inspection findings were reported to the Study Director on 15 October 1981. These inspections will also be included in the December 1981 report to management.

John C Johnson

JOHN C. JOHNSON
CPT, MS
Quality Assurance Officer

TABLE OF CONTENTS

THE MUTAGENIC POTENTIAL OF: 2,4-diamino-6-(2'-napthyl-sulfonyl) quinazoline

This test substance is a candidate anti-malarial drug tested by the Ames Assay at the request of the Walter Reed Army Institute of Research. The plate incorporation method was followed and the chemical was dissolved in DMSO. The DMSO was checked for sterility.

Rationale for using the Ames Assay

The Ames Salmonella/Mammalian Microsome Mutagenicity Test is one of a standard bank of tests used by our laboratory for the assessment of the mutagenic potential of a test substance. It is a short-term screening assay, which we use for the prediction of potential mutagenic agents in mammals. It is inexpensive when compared to in vivo tests, yet is highly predictive and reliable in its ability to detect mutagenic activity and therefore carcinogenic probability (1). It relies on basic genetic principles and allows for the incorporation of a mammalian microsome enzyme system to increase sensitivity through enzymatically altering the test substance into a potentially active metabolite. It has proven highly effective in assessing human risk (1).

Description of Test (Rationale for the selection of strains)

The test was developed by Bruce Ames, Ph.D. from the University of California-Berkeley. The test involves the use of several different genetically altered strains of Salmonella typhimurium, each with a specific mutation in the histidine operon (2). The test substance demonstrates mutagenic potential if it is able to revert the mutation in the bacterial histidine operon back to the wild type and thus reestablish prototrophic growth within the test strain. This reversion also can occur spontaneously due to a random mutational event. If, after adding a test substance, the number of revertants is significantly greater than the spontaneous reversion rate, then the test substance physically altered the locus involved in the operon's mutation and is able to induce point mutations and genetic damage (2).

In order to increase the sensitivity of the test system, two other mutations in the Salmonella are used (2). To insure a higher probability of uptake of test substance, the genome for the lipopolysacchride layer (LP) is mutated and allows larger molecules to enter the bacteria. Each strain has another induced mutation which causes loss of excision repair mechanisms. Since many chemicals are not by themselves mutagenic but have to be activated by an enzymatic process, a mammalian microsome system is incorporated. These microsomal enzymes are obtained from livers of rats induced with Aroclor 1254; the enzymes allow for the expression of the metabolites in the mammalian system. This activated rat liver microsomal enzyme homogenate is termed S-9.

Description of Strains (History of the strains used method to monitor the integrity of the organisms, and data pertaining to current and historical control and spontaneous reversion rates)

The test consists of using five different strains of Salmonella typhimurium that are unable to grow in absence of histidine because of a specific mutation in the histidine operon. This histidine requirement is verified by attempting to grow the tester strains on minimal glucose agar (MGA) plates, both with and without histidine. The dependence on this amino acid is shown when growth occurs only in its presence. The plasmids in strains TA 98 and TA 100 contain an ampicillin resistant R factor. Strains deficient in this plasmid demonstrate a zone of inhibition around an ampicillin impregnated disc. The alteration of the LP layer allows uptake by the Salmonella of larger molecules. If a crystal violet impregnated disc is placed onto a plate containing any one of the bacterial strains, a zone of growth inhibition will occur because the LP layer is altered. The absence of excision repair mechanisms can be determined by using ultraviolet (UV) light. These mechanisms function primarily by repairing photodimers between pyrimidine bases; exposure of bacteria to UV light will activate the formation of these dimers and cause cell lethality, since excision of these photodimers can not be made. The genetic mutation resulting in UV sensitivity also induces a dependence by the Salmonella to biotin. Therefore, this vitamin must be added. In order to prove that the bacteria are responsive to the mutation process, positive controls are run with known mutagens. If after exposure to the positive control substance, a larger number of revertants are obtained, then the bacteria are adequately responsive. Sterility controls are performed to determine the presence of contamination. Sterility of the test compound is also confirmed in each first dilution. Verification of the tester strains occurs spontaneously with the running of each assay. The value of the spontaneous reversion rate is obtained by using the same inoculum of bacteria that is used in the assay (3).

Strains were obtained directly from Dr. Ames, University of California-Berkeley, propagated and then maintained at -80 C in our laboratory. Before any substance was tested, quality controls were run on the bacterial strains to establish the validity of their special features and also to determine the spontaneous reversion rate (2). Records are maintained of all the data to determine if deviations from the set trends have occurred. These records are kept in the LAIR Archives.

In this series of tests for the detection of mutagenic potential of different agents, we compare the spontaneous reversion values with our own historical values and these cited by Ames et al (2). Our conclusions are based on the spontaneous reversion rate compared to the experimentally induced rate of mutation. When operating

effectively, these strains detect substances that cause base pair mutations (TA 1535, TA 100) and frameshift mutations (TA 1537, TA 1538, and TA 98).

METHODS (3)

Rationale for Dosage Levels and Dose Response Tabulations

To insure readable and reliable results, a sublethal concentration of the test substance had to be determined. This toxicity level was found by using MGA plates, various concentrations of the substance, and approximately 10^8 cells of TA 100 per plate, unless otherwise specified. Top agar containing trace amounts of histidine and biotin were placed on MGA plates. TA 100 is used because it is the most sensitive strain. Strain verification was confirmed on the bacteria, along with a determination of the spontaneous reversion rate. After incubation, the growth was observed on the plates. (The auxotrophic Salmonella will replicate a few times and potentially express a mutation. When the histidine and biotin supplies are exhausted, only those bacteria that reverted to the prototrophic phenotype will continue to reproduce and form macrocolonies; the remainder of the bacteria comprises the background lawn. The minimum toxic level is defined as the lowest serial dilution at which decreased macrocolony formation, below that of the spontaneous revertant rate, and an observable reduction in the density of the background lawn occurs.) A maximum dose of 1 mg/plate is used when no toxicity is observed. The densities were recorded as normal, slight, and no growth.

Test Format

After we validated our bacterial strains and determined the optimal dosage of the test substance, we began the Ames Assay. In the actual experiment, 0.1 ml of the particular strain of Salmonella (10^8 cells) and the specific dilutions of the test substance are added to 2 ml of molten top agar, which contained trace amounts of histidine and biotin. Since survival is better from cultures which have just passed the log phase, the Salmonella strains are used 16 hours (maximum) after initial inoculation into nutrient broth. The dose of the test substance spanned a 1000-fold, decreasing from the minimum toxic level by a dilution factor of 5. All the substances were tested with and without S-9 microsome fraction. The optimal titer of the S-9 was determined and 0.5 ml was added to the molten top agar. After all the ingredients were added, the top agar was mixed, then overlaid on minimum glucose agar plates. These plates contained 2% glucose and Vogel Bonner "E" Concentrate (4). The water used in this medium and all reagents came from a polymetric system. Plates were incubated, upside down in the dark at 37 C for 48 hours. Plates were prepared in triplicate and the average revertant counts were recorded. The

corresponding number of revertants obtained was compared to the number of spontaneous revertants; the conclusions were recorded statistically. A correlated dose response is considered necessary to declare a substance as a mutagen. Commoner (5), in his report, "Reliablilty of Bacterial Mutagenesis Techniques to Distinguish Carcinogenic and Non-Carcinogenic Chemical," and McCann et al (1) in their paper, "Detection of Carcinogens as Mutagens in the Salmonella/Mammalian Microsome Mutagenicity Test: Assay of over 300 Chemicals," have concurred on the test's ability to detect mutagenic potential.

Statistical Analysis

Quantitative evaluation was ascertained by the method of Ames (2). He assumes that a chemical which caused twice the revertant rate experimentally as spontaneously, is mutagenic.

Chemical Analysis

Our information on the chemical analysis of the test compound was obtained from Chun and Lam (Appendix A), WR 158,122 · Free Base, Lot AC, Bottle Number AY65859.

RESULTS AND DISCUSSION

Throughout this report, 2,4-diamino-6-(2'-napthyl-sulfonate) quinazoline will be referred to by its code number WR158122.

On 1 October 1981, the Toxicity Level Determination was performed on the test substance. For this experiment, all sterility, strain verification, positive and negative controls were normal (Table 1). Toxicity was observed at the three highest doses. It was decided to use 10^{-3} mg/plate as the initial dose.

Two Ames Assays were run to determine conclusively the mutagenic activity of WR158122. The initial assay performed on 6 October 1981 showed normal results to all strain verification and sterility controls (Table 3). An unexpected response was observed for the spontaneous reversion rates taken at the end of the assay. An unexpected response by TA 1538 to positive control chemical dimethyl benzanthracene (DMBA) was also observed (Table 4). After exposure of the bacteria to the test chemical, there were instances of no growth and abnormally low reversion rates. No mutagenicity was observed (Table 5). Due to the irregular results, a second assay was run on 22 October 1981.

In the second experiment, all sterility and strain verification controls were normal (Table 6). The spontaneous reversion rate and all positive controls were normal except the response of TA 1538 to DMBA (Table 7). In response to the test chemical, there was no evidence of mutagenicity (Table 8).

CONCLUSION

The Ames Test is able to detect frameshift and basepair mutagenic potential. Our results show no evidence of such potential. Therefore, on the basis of the Ames test, WR158122, both in the presence and absence of metabolic activation, is not mutagenic at the levels tested.

RECOMMENDATION

2,4-diamino-6-(2'-napthyl-sulfonyl) quinazoline should be tested using other toxicological assays if efficacy tests prove this compound to be a promising anti-malarial drug.

REFERENCES

1. McCANN, J., E. CHOI, E. YAMASAKI, and B. N. AMES. Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc Nat Acad Sci, USA 72:5135-5139, 1975

2. AMES, B. N., J. McCANN and E. YAMASAKI. Methods for detection carcinogens and mutagens with Salmonella/mammalian microsome mutagenicity test. Mutation Res 31: 347-364, 1975

3. LAIR SOP OP-STX-1, Ames Salmonella/mammalian microsome mutagenicity test, 1 March 1981

4. VOGEL, H. J. and D. M. BONNER. Acetylornithinase of E. coli: Partial purification and same properties, J Biol Chem 218: 97-106, 1956

5. COMMONER, B. Reliability of the bacterial mutagenesis techniques to distinguish carcinogenic and non-carcinogenic chemicals. EPA 600/1 76-022, 1976

Report on Chemical Analysis

APPENDIX A

APPENDIX A

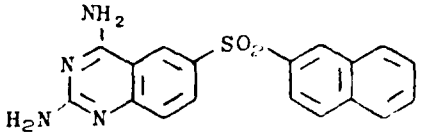
STANFORD RESEARCH INSTITUTE
Life Sciences Division
Contract No. DA-49-193-MD-2753
January 7, 1972

Report No. 202

Sample: WR-153122 AC; AY 65859

2,4-Diamino-6-(2-naphthylsulfonyl)quinazoline

$C_{18}H_{14}N_4O_2S$ 350.40



This lot of 2,4-diamino-6-(2-naphthylsulfonyl)quinazoline contains not less than 94.7% of $C_{18}H_{14}N_4O_2S$ according to a titration assay. The mass spectrum indicates an impurity with a mass of 369 and tlc shows two minor contaminants amounting to ~ 1%. The rest of the contaminants in the sample is mainly water. The stability results will be reported as soon as they become available.

Discription: Light tan material composed of aggregates and powder.

Melting Range: (capillary, applied at 300°)

"As received": 325-327° dec.
Dried 100°, 1 mm, 5.5 hr: 328-330 dec.

Loss on Drying: (100°, 1 mm Hg, 5.5 hr.) 4.6% from a 258.1-mg sample.

Karl Fischer Water Determination: 5.6 ± 0.6% (two determinations)

Residue on Ignition: (950°, O_2) 0.12% from a 16.027-mg sample.

Identity

A. An infrared absorption spectrum, recorded as a Nujol mull, exhibits maxima that are compatible with the structural formula. Assignments are found on the spectrum (Fig. 1).

B. The ultraviolet absorption spectrum of a 1 in 125,000 Methyl Cellosolve solution exhibits maxima at the following wavelengths:

λ, nm	ε
236.5	59,800
311	31,900

APPENDIX A, continued

Assay: Perchloric acid titration [Anal. Chem. 24, 300 (1952)]. Accurately weighed portions (~ 180 mg) of the sample were dissolved in 80 ml of glacial acetic acid with the aid of sonication. The solutions were titrated with 0.1N acetous perchloric acid (standardized against potassium acid phthalate, NBS) on a Sargent recording titrator using glass and calomel electrodes. The result is the average of four determinations.

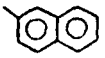
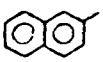
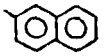
	Equivalent Weight	Equivalent % Purity
Theoretical	350.4	
Found	370.1 ± 0.8	94.7 ± 0.2

Victoria Chun
Victoria Chun
Technician

Allen Benitez
Allen Benitez
Chemist

Peter Lim
Peter Lim
Organic Chemist

C. The nuclear magnetic resonance spectrum (Fig. 2) run in DMSO-d_6 is in agreement with the structural formula.

D. The mass spectrum indicates a molecular ion of 350, which is in agreement with the compound. Also present is an impurity (M = 469) with a major fragmentation at 391. Other major fragmentations are 331 ($M-NH_2-3H$), 313 ($M-2NH_2-H$), 286 ($M-2NH_2-20$), 207 (M-  $-NH_2$), 191 ( SO_2), 175 (M-  $-2NH_2-O$), 159 (M-  SO_2) and 127 ().

Solubility: (constant stirring at r.t. for 1 hr) Very soluble in dimethylsulfoxide (> 100 mg/ml); insoluble in propylene glycol/ethanol/water containing 0.9% (by weight) NaCl (1/1/3, v/v/v) and in water containing 0.9% (by weight) NaCl.

Elemental Analysis: Calculated for $C_{18}H_{14}N_4O_2S$

	C	H	N	S
Calcd.	61.70	4.03	15.99	9.15
Found	58.66	4.18	15.44	8.71

The elemental data suggest a 95-97% purity. A sample that was dried at 100°, 1 mm for 5.5 hours and showed a loss of 4.6% analyzed for C, 59.50; H, 4.16; N, 15.51 and was reported by the analyst to be quite hygroscopic.

Thin-Layer Chromatography

Adsorbent: SiO_2-HF
Quantities applied: 25, 50, 75 γ (25 γ/λ, MeCell)
Detection: uv and iodine vapor
Solvent systems: a. Dioxane/$CHCl_3$ (9/1, v/v)
b. EtOAc/HOAc (4/1, v/v)
c. 1,2-Dimethoxyethane (plate prewashed in solvent)
Results: In solvent system (a) the material is resolved into two spots--R_f 0.00 (minor) and 0.78 (WR-158122, major). In solvent system (b) the material displays a trace spot at the origin and a major spot at R_f 0.51. Systems (a) and (b) overload very easily which results in elongation of the major spot. In solvent system (c) the material is resolved into two minor spots at R_f 0.01 (~ 0.3%*) and 0.12 (~ 0.7%*) and a major spot (WR-158122) at R_f 0.39. A two-dimensional plate proved the two minor contaminants to be real. If the plate is not prewashed, then occasionally two more minor spots appear at R_f 0.73 and 0.87. These two spots could not be proved real by two-dimensional tlc because once the plate has been run through the solvent, the major spot at R_f 0.39 and the two spots at R_f 0.73 and 0.87 run at the same rate.

*Quantitations are based on the assumption that the same amount of each spot shows comparable fluorescence and responds comparably to iodine vapor.

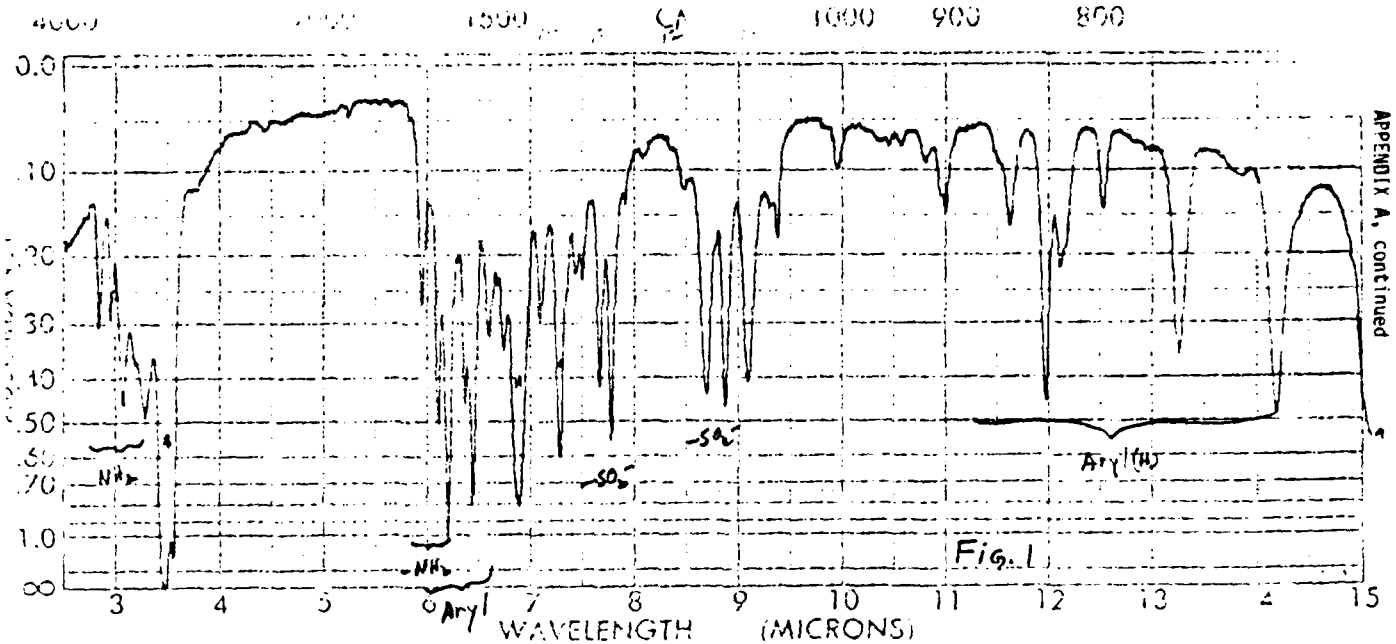
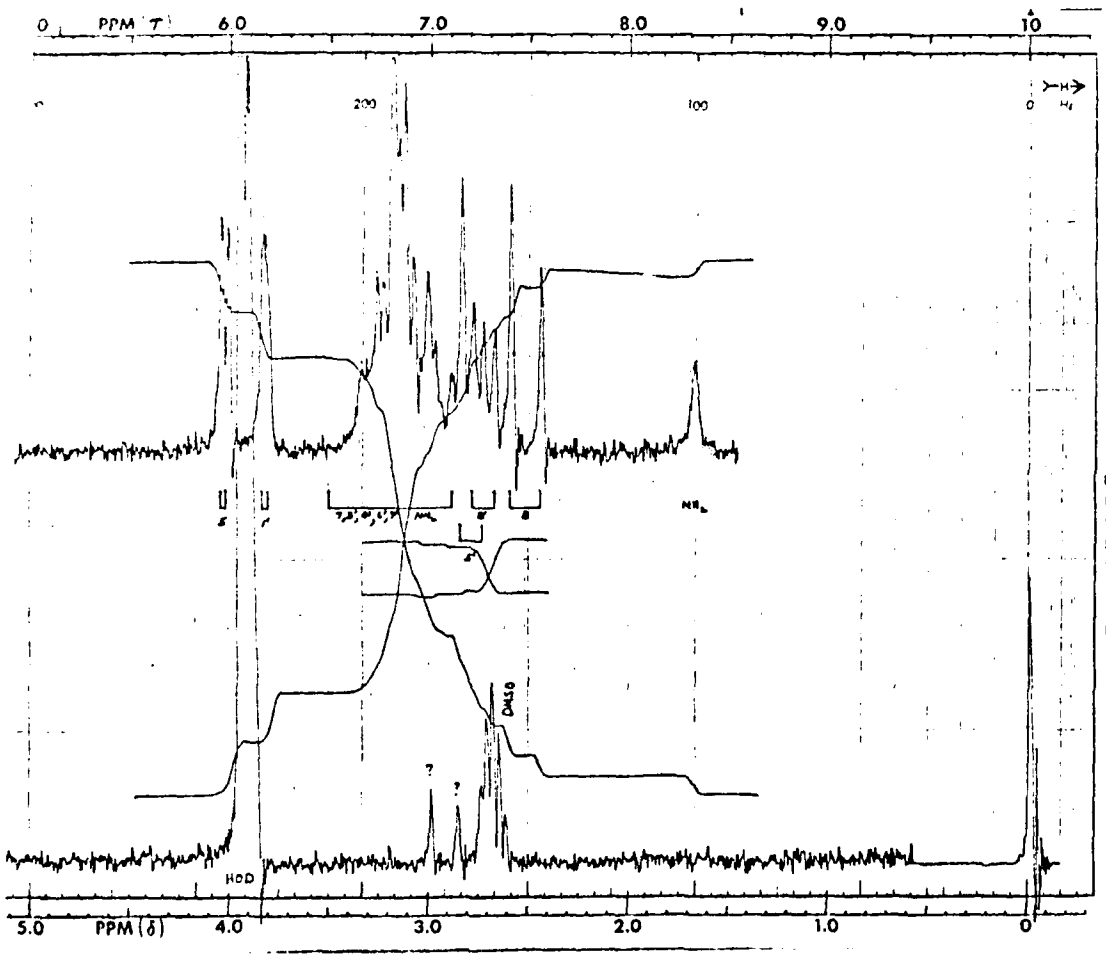


Fig. 1

SPECTRUM NO.	ORIGIN	LEGEND	REMARKS
SAMPLE WR-158122 AC		1. * Nujol	
	PURITY	2.	
	PHASE Nujol mull	DATE Sept. 2, 1971	
	THICKNESS Thin film	OPERATOR A. Benitez	

SAMPLE



60 MHz NMR SPECTRUM NO. A2 B-Nitro2

OPERATOR Cruse DATE 8/18/71

SAMPLE WR: 158/22 AC

SOLVENT	D6 DMSO		
TEMPERATURE	4		°C
FILTER BANDWIDTH	4		Hz
R.F. FIELD	.04	.05	mG
SWEEP TIME	250	50	sec
SWEEP WIDTH	500		Hz
SWEEP OFFSET	300		Hz
SPECTRUM AMP.	8.0	2.0	
INTEGRAL AMP.		30.0	

REMARKS: D2O Exchanged (int. refs.)

Fig 2

VARIAN CHART 540-C

APPENDIX A, continued

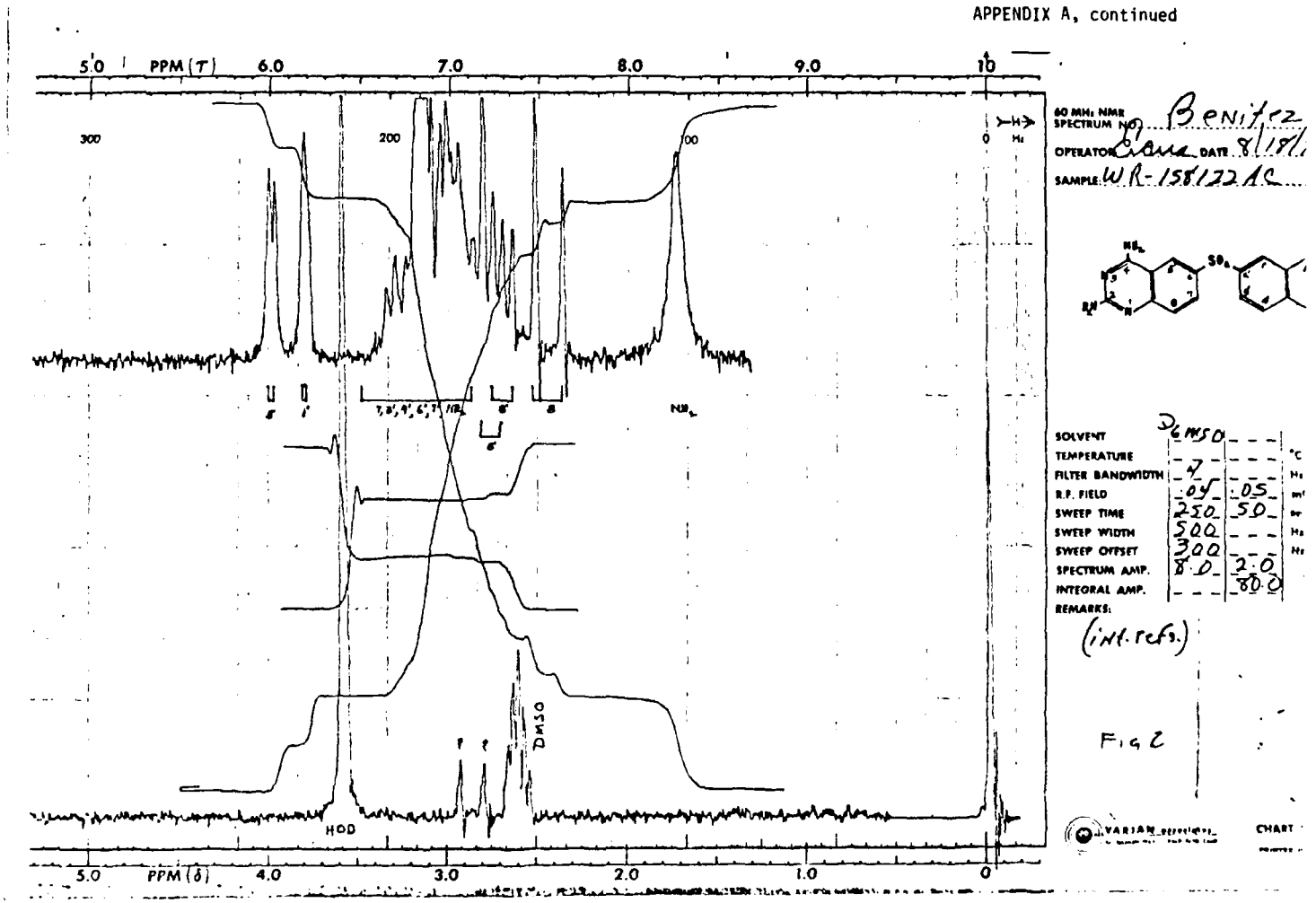
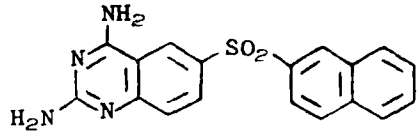


Fig 2

STANFORD RESEARCH INSTITUTE
Life Sciences Division
Contract No. DA-49-193-MD-2753
September 12, 1972

Addendum to Report No. 202

Sample: WR-158122 AC, AY 65859



2,4-Diamino-6-(2'-naphthylsulfonyl)quinazoline

$C_{18}H_{14}N_4O_2S$ 350.4

A.	Material	stored	at	r.t.	for 1 month
B.	"	"	"	"	" 3 months
C.	"	"	"	45°	" 2 weeks
D.	"	"	"	"	" 1 month
E.	"	"	"	"	" 2 months
F.	"	"	"	"	" 3 months
G.	"	"	"	60°	" 1 week
H.	"	"	"	"	" 2 weeks
I.	"	"	"	"	" 1 month
J.	"	"	"	"	" 2 months

The objective of this investigation was to determine the stability of the chemical after it had been stored as required. All evidence indicates that the chemical is stable under the conditions of the stability study.

Experimental and Discussion

The materials that were stored at r.t. for 3 months, 45° for 3 months, and 60° for 2 months were examined by infrared (Fig. 1, typical spectrum), ultraviolet (Fig. 2, typical spectrum), nmr (Fig. 3, typical spectrum) and tlc. All data indicate no decomposition.

Thin-Layer Chromatography

Adsorbent: SiO_2-HF

Quantities applied: 20, 60, 80 γ (20 γ/λ, MeCell)

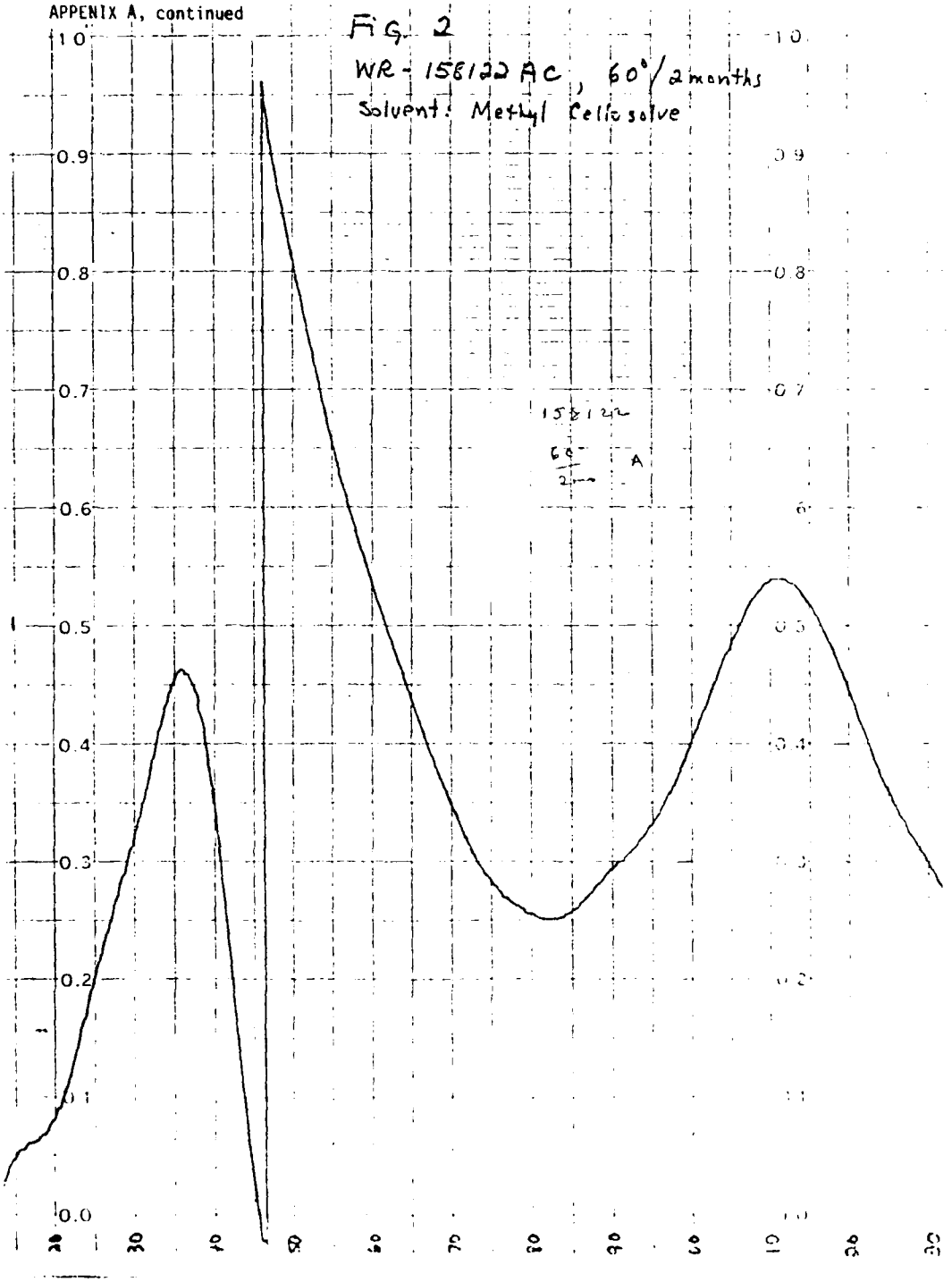
Reference: WR-158122 AC, AY 65859 (SRI Report No. 202)

Detection: uv, iodine vapor

Solvent systems:
a. dioxane/$CHCl_3$ (9/1, v/v)
b. EtOAc/HOAc (4/1, v/v)
c. 1,2-dimethoxyethane (plate prewashed with solvent)

Results: The three samples are chromatographically identical to the reference in all three solvent systems--(a) R_f 0.00 (minor) and R_f 0.71 (major); (b) R_f 0.00 (trace) and R_f 0.48 (major); and (c) R_f 0.00 (minor), R_f 0.10 (minor) and R_f 0.35 (major).

Fig. 2
WR-158122 AC, 60°/2 months
Solvent: Methyl Cellosolve



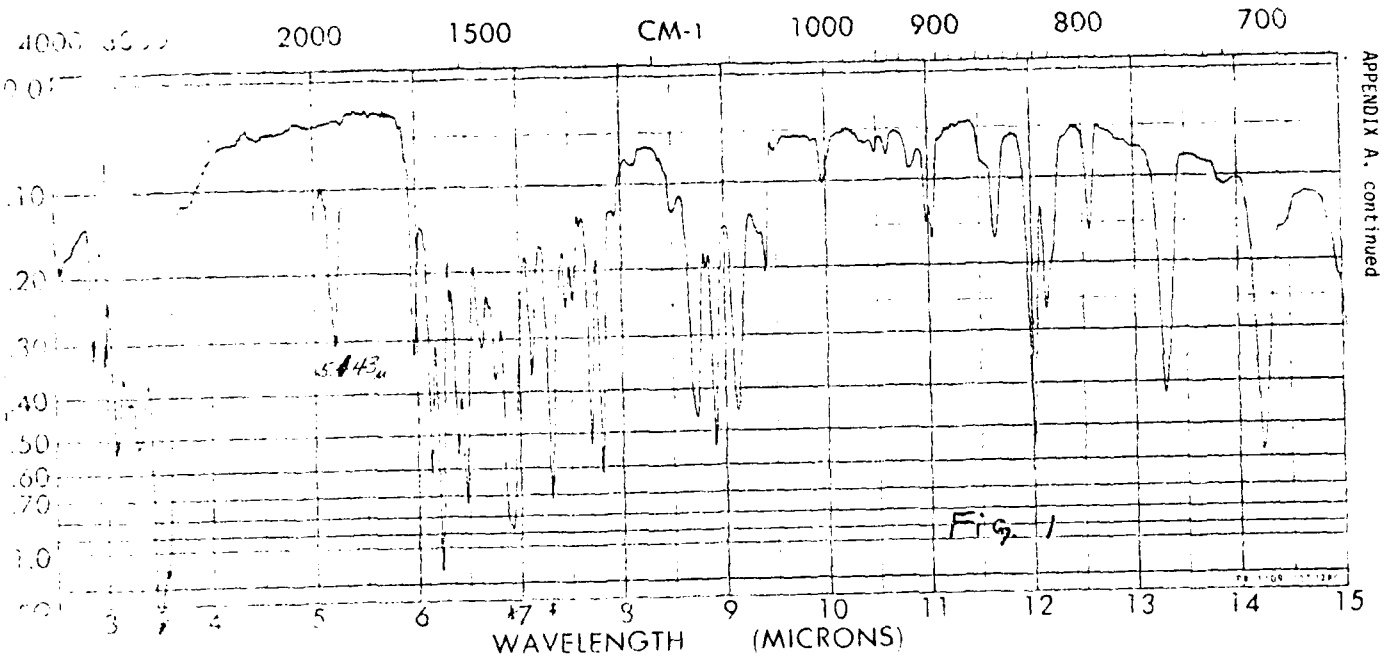


Fig. 1

SPECTRUM NO	ORIGIN	LEGEND	REMARKS
SAMPLE AC - 60/2mm		1 * Nujol	
	PURITY	2	
	PHASE Nujol	DATE 5/8/61	
	THICKNESS	ORDINATE	

SAMPLE

APPENDIX A, continued

Ultraviolet Spectra (Methyl Cellosolve)

The ultraviolet spectra of samples A through J are all similar. The maxima and the molar absorptivities for the individual samples are as follows:

	ϵ*	
Sample	λ_{max} 236.5 nm	λ_{max} 311 nm
A	61,500	22,600
B	61,100	22,500
C	61,500	22,500
D	61,100	22,500
E	61,100	22,500
F	61,400	22,800
G	61,300	22,500
H	61,200	22,500
I	61,300	22,700
J	61,100	22,500
Reference (WR-158122 AC, AY 65859, SRI Report No. 202)	61,300	22,600

Michael Lam PL
Michael Lam
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Allen Benitez
Allen Benitez
Chemist

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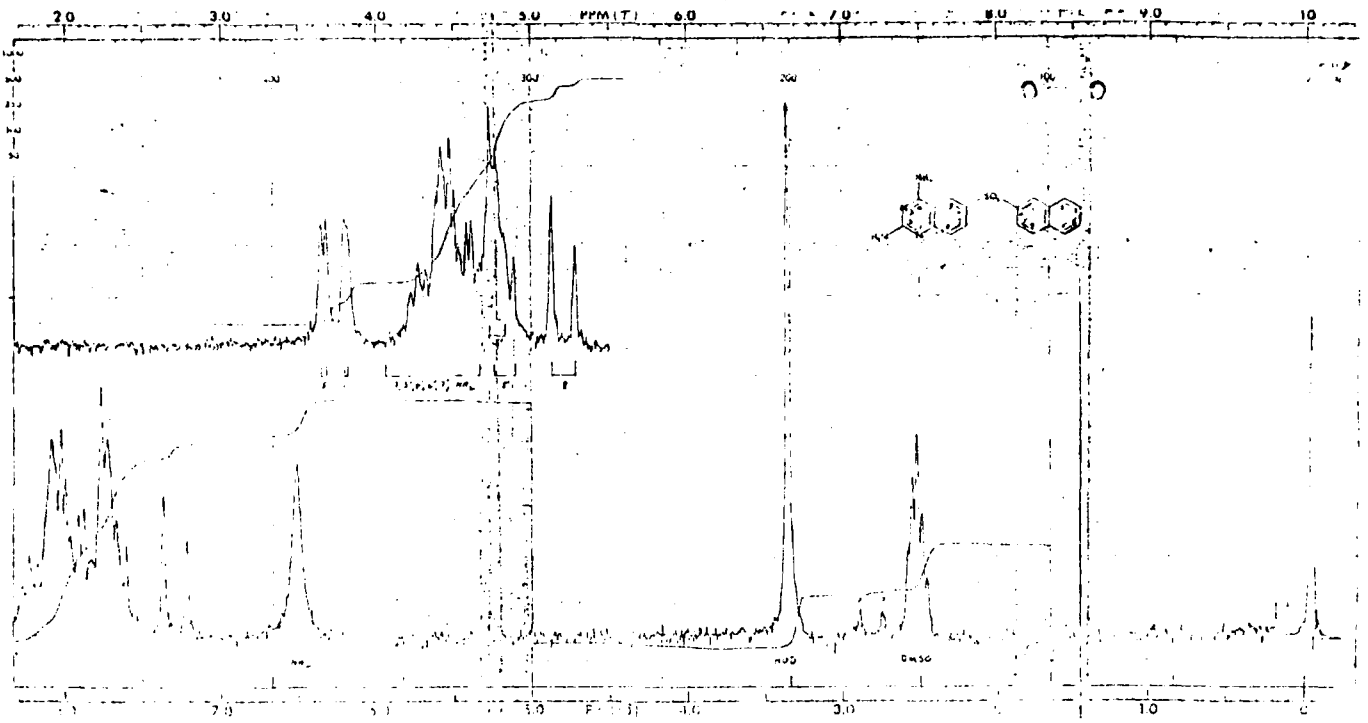
Peter Lim
Organic Chemist

*Average of two determinations.

APPENDIX A, concluded



LIST OF TABLES

APPENDIX B

TABLE 1

STRAIN VERIFICATION FOR TOXICITY LEVEL DETERMINATION

Strains	Histidine Requirement	Ampicillin Resistance	UV Sensitivity to Crystal Violet		Sterility Control	Response (1)
100	NG	G	NG	14.11 mm	NG	+
1537	NG	14.11 mm	NG	13.86 mm	NG	+
WT	G	NA	G	NA	NA	+

STERILITY CONTROL

His-Bio Mix Initial: NG End: NG MGA Plate: NG

Top Agar Initial: NG End: NG

Diluent: NG Nutrient Broth: NG

Test Compound (a) Chloroquine NG (b) WR158122 NG (c) NA (d) NA (e) NA

G = Growth NG = No Growth NT = Not Tested NA = Not Applicable WT = Wild Type

Spontaneous Revertants: TA 100, No S-9 , average = 79

(1) + = expected response - = unexpected response

Study Number: 81031/81032 Date: 1 Oct 81 By: Sauers

TABLE 2

TOXICITY LEVEL DETERMINATION

Substance assayed: WR 158122 Substance dissolved in: DMSO

Study Number: 81032 Date: 1 Oct 81 Performed by: Sauers, Mullen, Dacey

TA 100 REVERTANT PLATE COUNT

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	Background Lawn (1)
1 mg/plate	TOXIC	TOXIC	TOXIC	TOXIC	NG
10^{-1} mg/plate	TOXIC	TOXIC	TOXIC	TOXIC	NG
10^{-2} mg/plate	TOXIC	TOXIC	TOXIC	TOXIC	NG
10^{-3} mg/plate	56	62	71	63	NL
10^{-4} mg/plate	66	67	59	64	NL
10^{-5} mg/plate	85	73	72	77	NL
10^{-6} mg/plate	59	49	68	59	NL
10^{-7} mg/plate	71	73	69	71	NL

(1) NG = No Growth ST = Slight Growth NL = Normal Lawn

TABLE 3

STRAIN VERIFICATION CONTROL

Strains	Histidine Requirement	Ampicillin Resistance	UV	Sensitivity to Crystal Violet	Sterility Control	Response (1)
98	NG	G	NG	14.13 mm	NG	+
100	NG	G	NG	14.89 mm	NG	+
1535	NG	NA	NG	13.56 mm	NG	+
1537	NG	16.11 mm	NG	13.87 mm	NG	+
1538	NG	NA	NG	13.93 mm	NG	+
WT	G	NA	G	NA	NA	+

STERILITY CONTROL

His-Bio Mix Initial: NG End: NG Diluent: NG

Top Agar Initial: NG End: NG MGA Plate: NG

S-9 Mix Initial: NG End: NG Nutrient Broth: NG

Test Compound (a) Chloroquine NG (b) WR158122 NG (c) NA (d) NA (e) NA (f) NA

G = Growth NG = No Growth NT = Not Tested NA = Not Applicable WT = Wild Type

Study Number: 81031/81032 By: Sauers, Mullen, Dacey, Kellner

Date: 6 Oct 81

(1) + = expected response
- = unexpected response

TABLE 4

QUALITY AND POSITIVE CONTROLS (number of revertants/plate)

Compd.	Amount of Compd. Added	S-9 Added	Strain No. 98	100	1535	1537	1538
AF	2 ug/plate	yes	(329,265,288) 294	(160,283,235) 226			(189,310,383) 294
BP	2 ug/plate	yes	(141,138,225) 168	(177,197,171) 182		(69, 44,106) 73	(44, 38, 78) 53
DMBA	20 ug/plate	yes	(36, 30, 42) 36	(87, 93,107) 96		(12, 8, 12) 11	(24, 19, 10) 18
MNNG	2 ug/plate	no		(446,498,413) 452			
	20 ug/plate	no			(311,309,352) 324		
Spontaneous Reversion Rate							
	before	no	(20, 14, 9)	(62, 65, 64)	(7, 6, 10)	(4, 2, 7)	(8, 14, 7)
	after		(NG, NG, NG) 14	(NG, NG, NG) 64	(NG, NG, NG) 8	(NG, NG, NG) 4	(NG, NG, NG) 10
	before	yes	(14, 14, 9)	(66, 79, 74)	(10, 13, 8)	(2, 4, 5)	(7, 5, 7)
	after		(NG, NG, NG) 12	(NG, NG, NG) 73	(NG, NG, NG) 10	(NG, NG, NG) 4	(NG, NG, NG) 6

NG = no growth

Study Number: 81031/81032

Date: 6 Oct 81 By: Sauers, Dacey, Kellner, Mullen

TABLE 5

SALMONELLA/MICROSOME ASSAY WORKSHEET (number of revertants/plate)

Compd	Amount of Compd. Added	S-9 Added	Strain No. 98	100	1535	1537	1538
WR158122	10^{-3} mg/pl	no	(6, 3, 2) 4	(21, 9, 3) 11	(4, 5, 3) 4	(2, NG, NG) 2	(6, 6, 5) 6
		yes	(3, 7, 6) 5	(15, 10, NG) 13	(2, 4, 5) 4	(2, 3, 3) 3	(17, 16, 13) 15
	$2x10^{-4}$ mg/pl	no	(4, 3, 3) 3	(56, 43, 37) 45	(5, 6, 4) 5	(2, 5, 3) 3	(7, 6, 11) 8
		yes	(NG, NG, NG) (NG)	(45, 50, 38) 44	(2, 3, 2) 2	(4, 2, 3) 3	(15, 15, 7) 12
	$4x10^{-5}$ mg/pl	no	(NG, NG, NG) (NG)	(50, 50, 29) 43	(8, 4, 3) 5	(3, 2, 2) 2	(5, 6, 7) 6
		yes	(NG, NG, NG) (NG)	(18, 16, 27) 20	(2, 7, 5) 5	(3, NG , NG) 3	(5, 4, 6) 5
	$8x10^{-6}$ mg/pl	no	(NG, NG, NG) (NG)	(53, 37, 25) 38	(5, 6, 3) 5	(2, 2, 1) 2	(17, 12, 6) 12
		yes	(NG, NG, NG) (NG)	(40, 34, 39) 38	(9, 8, 2) 6	(4, 2, 3) 3	(15, 5, 7) 9

-continued

Study No.: 81032 Date: 6 Oct 81 Performed by: Sauers, Kellner, Dacey, Mullen

TABLE 5, concluded

SALMONELLA/MICROSOME ASSAY WORKSHEET (number of revertants/plate)

Compd	Amount of Compd. Added	S-9 Added	Strain No. 98	100	1535	1537	1538
WR158122	1.6×10^{-6} mg/pl	no	(NG, NG, NG) (NG)	(NG, NG, NG) (NG)	(NG, NG, NG) (NG)	(NG, NG, NG) (NG)	(NG, NG, NG) (NG)
		yes	(21, 13, 13) 16	(58, 46, 57) 54	(15, 6, 9) 10	(4, 3, 2) 3	(NG, NG, NG) (NG)
	3.2×10^{-7} mg/pl	no	(NG, NG, NG) (NG)	(54, 44, NG) 49	(9, 10, 8) 9	(2, 2, 3) 2	(9, 5, 6) 7
		yes	(NG, NG, NG) (NG)	(25, 10, 20) 18	(4, 2, 1) 2	(3, 2, 2) 2	(6, 3, 7) 5

NG = no growth

Study No.: 81032 Date: 6 Oct 81 Performed by: Sauers, Kellner, Dacey, Mullen

TABLE 6

STRAIN VERIFICATION CONTROL

Strains	Histidine Requirement	Ampicillin Resistance	UV	Sensitivity to Crystal Violet	Sterility Control	Response (1)
98	NG	G	NG	13.94 mm	NG	+
100	NG	G	NG	14.06 mm	NG	+
1535	NG	NA	NG	13.56 mm	NG	+
1537	NG	15.89 mm	NG	12.89 mm	NG	+
1538	NG	NA	NG	13.21 mm	NG	+
WT	G	NA	G	NA	NA	+

STERILITY CONTROL

His-Bio Mix Initial: NG End: NG Diluent: NG

Top Agar Initial: NG End: NG MGA Plate: NG

S-9 Mix Initial: NG End: NG Nutrient Broth: NG

Test Compound Chloroquine WR158122 (a) NG (b) NG (c) NA (d) NA (e) NA (f) NA

G = Growth NG = No Growth NT = Not Tested NA = Not Applicable WT = Wild Type

Study Number: 81031/81032 By: Sauers

Date: 21 Oct 81

(1) + = expected response
- = unexpected response

TABLE 7

QUALITY AND POSITIVE CONTROLS (number of revertants/plate)

Compd.	Amount of Compd. Added	S-9 Added	Strain No. 98	100	1535	1537	1538
AF	2 ug/plate	yes	(493,506,488) 496	(476,446,311) 411			(677,677,545) 633
BP	2 ug/plate	yes	(83,125,115) 108	(485,523,439) 482		(62, 78, 84) 75	(83, 84,115) 94
DMBA	20 ug/plate	yes	(51, 44, 74) 56	(244,277,245) 255		(15, 17, 24) 19	(27, 22, 22) 24
MNNG	2 ug/plate	no		(999,999,999) * 999			
	20 ug/plate	no			(999,999,999) * 999		
Spontaneous Reversion Rate							
	before	no	(20, 28, 25)	(98, 89, 96)	(15, 13, 14)	(7, 9, 6)	(17, 12, 11)
	after		(16, 24, 22) 22	(104,123,101) 102	(12, 15, 17) 14	(6, 7, 9) 7	(11, 12, 15) 13
	before	yes	(27, 22, 20)	(95, 80, 94)	(11, 12, 11)	(5, 6, 2)	(12, 15, 16)
	after		(18, 29, 26) 24	(117,121,126) 106	(12, 19, 22) 14	(4, 6, 7) 5	(19, 17, 26) 18

* A value of 999 represents a count of over 1000

Study Number: 81031/81032

Date: 22 Oct 81 By: Sauers, Dacey, Kellner, Mullen

TABLE 8

SALMONELLA/MICROSOME ASSAY WORKSHEET (number of revertants/plate)

Compd	Amount of Compd. Added	S-9 Added	Strain No. 98	100	1535	1537	1538
WR158122	10^{-3} mg/pl	no	(13, 9, 22) 15	(63, 85, 71) 73	(12, 9, 11) 11	(2, 3, 4) 3	(11, 9, 16) 12
		yes	(22, 16, 9) 16	(52, 56, 45) 51	(13, 7, 13) 11	(4, 5, 7) 5	(19, 16, 22) 19
	$2x10^{-4}$ mg/pl	no	(16, 17, 9) 14	(80, 74,100) 85	(14, 13, 12) 13	(5, 6, 3) 5	(12, 11, 11) 11
		yes	(12, 22, 20) 18	(137,103, 97) 112	(11, 8, 6) 8	(7, 3, 3) 4	(29, 13, 28) 23
	$4x10^{-5}$ mg/pl	no	(17, 27, 16) 20	(107, 94,117) 106	(19, 17, 6) 14	(5, 3, 3) 4	(19, 18, 15) 17
		yes	(27, 18, 35) 27	(105, 97, 91) 98	(19, 11, 17) 16	(4, 3, 8) 5	(16, 17, 19) 17
	$8x10^{-6}$ mg/pl	no	(17, 15, 24) 19	(106,101,102) 103	(16, 16, 19) 17	(4, 7, 8) 6	(12, 11, 18) 14
		yes	(34, 29, 28) 30	(100,121, 73) 98	(23, 30, 28) 27	(5, 6, 5) 5	(27, 19, 22) 23

-continued

Study No.: 81032 Date: 22 Oct 81 Performed by: Sauers, Dacey, Kellner, Mullen

TABLE 8, concluded

SALMONELLA/MICROSOME ASSAY WORKSHEET (number of revertants/plate)

Compd	Amount of Compd. Added	S-9 Added	Strain No. 98	100	1535	1537	1538
WR158122	1.6×10^{-6} mg/pl	no	(22, 20, 20) 21	(86, 97, 83) 89	(24, 19, 13) 19	(4, 9, 7) 7	(16, 15, 9) 13
		yes	(18, 23, 26) 22	(90, 81, 78) 83	(16, 3, 5) 8	(4, 8, 8) 7	(15, 14, 20) 16
	3.2×10^{-7} mg/pl	no	(24, 9, 15) 16	(70, 74, 71) 72	(17, 12, 19) 16	(3, 5, 5) 4	(8, 11, 13) 11
		yes	(24, 23, 30) 26	(78, 91, 85) 85	(7, 5, 8) 7	(6, 5, 9) 7	(27, 19, 23) 23

Study No.: 81032 Date: 22 Oct 81 Performed by: Sauers, Dacey, Kellner, Mullen

APPENDIX C: LAIR-SOP-OP-STX-1

LAIR SOP-OP-STX-1 (1 May 1981)

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